

**FY08 Application for Nursery Research Funding**  
**Washington State Department of Agriculture - Nursery License Surcharge**  
 (Please use one application packet including the Progress Report page for each proposal.  
 You must use our form - failure to do so may result in not funding your project.)

Project Title: Science-based strategies for mitigating the impact of viruses in perennial nursery crops

Project Leader: Naidu A Rayapati

Institution (if any): Washington State University

Mailing Address: IAREC, 24106 N. Bunn Road, Prosser, WA 99350

Project Phone Number: (509)786-9215 FAX Number: (509)786-9370 Cellular/Pager Number: (509)781-0665

Note: Project leader or his/her designee must be available at above project phone number on March 2, 2007 between the hours of 10:00-12:00 and 1:00-3:00.

Amount Requested for (FY08) July 1, 2007 to June 30, 2008: \$14,503

Start Date: July 1, 2007 Completion Date: June 30, 2010

(Check One) New Project  Continuing

If this is a multiple year project, please estimate and list the following information for each future July 1 - June 30 period listed below through project completion:

Fiscal Years (FY)	July 1, 2008 to June 30, 2009	July 1, 2009 to June 30, 2010	July 1, 2010 to June 30, 2011	July 1, 2011 to June 30, 2012	July 1, 2012 to June 30, 2013
\$ Amount Needed	14,709	14,922			

If you are increasing the above amounts since your last application, please explain why:

\*Please list all other sources and amounts of funding for this project for the current year only: (Please notify us by February 15 if other funding has been approved and from where.)

Source	\$ Amount Applied For	Approved	Pending Date of Notification
IPM-CRSP (USAID)	\$19,485	Yes	
In-Kind from Nurseries	\$3,000	Yes	

Total Amount Needed to Fund Project (Include all sources) \$ 36,988

If total amount from all sources is not granted, will you be able to complete the project? Partially  
 Explain:

A Visiting Scientist supported by IPM CRSP (salary and benefits for six months) will participate in this project. In-kind from nurseries include personnel time and plant materials supplied for research. If funding is limited, the number of proposed activities of the project will be scaled down. This will lead to slow progress of research and impact research outputs reaching the nurseries in a timely manner. Nursery License Surcharge will also serve as a catalyst for soliciting additional funding from sources like Washington State Commission on Pesticide Registration and USDA-ARS Northwest Nursery Crop Research Center during 2007/2008 cycle.

Submit 15 copies of this proposal to: Tom Wessels, Plant Services Program Manager, P.O. Box 42560, Olympia, WA 98504-2560. All applications must be postmarked by December 31, 2006.

**Please summarize the purpose of this research: (you may attach additional sheets if necessary or submit this summary in your own format)**

According to an economic impact analysis conducted by Washington State University School of Economic Sciences (Working Paper Series WP 2006-1), the Green Industry comprising growers of Nursery, sod, and floriculture crops generated \$2.48 billion of total sales and contributed \$1.73 billion of value-added income to the Washington State economy during the year 2005. The study also estimated that the Green Industry creates, either directly or indirectly, about 43,000 jobs. Thus, Nursery and Floriculture industries play a vital role in the economy of Washington State.

Virus diseases are arguably among the most serious impediments to the sustainability of the Nursery/Floriculture industry in Washington State. The recent emergence of viruses like Hosta Virus X in nursery crops in several states of the U.S.A. demonstrates the vulnerability of the nursery industry to many exotic and emerging virus disease problems and stresses the need for continuous vigilance to guard the nursery industry against viruses. Once introduced, viruses can spread throughout any suitable ecosystem available to them, regardless of national or state boundaries. Consequently, viruses can cause revenue loss in several ways, including disfiguring foliage and flowers resulting in plants with poor horticultural aesthetics, reducing plant vigor, and reducing the life span either in the nursery or in the landscape setting. With the greenhouse and bedding plant industries facing intense competition, maintenance and supply of virus-free plants is vital to retain competitive advantage for the Nursery/Floriculture industry in the State. Given the fact that viral diseases cannot be controlled by chemical agents analogous to fungicides and bactericides, they must instead be controlled by phytosanitary measures. Such measures include growing virus-free plants, preventing the introduction of viruses through importing infected planting stock and targeting insect vectors that spread many debilitating virus diseases.

Many viruses are systemic and invade the entire plant. Thus, once a plant is infected, it remains infected throughout its life. Symptom expression in virus-infected plants is dependent on many variables, including seasonal changes and growth stage of the plant. Consequently, many perennial, vegetatively propagated plants grown by the nursery industry as stock plants in the greenhouse or field settings may carry the virus without showing obvious symptoms and spread from one crop to the next when such plants are used as the source of cuttings. In addition, virus infected perennial stock plants in the field will become a constant source of virus for other crops. In many situations, perennial stock plants propagated in the field are continuously challenged by infection with new viruses spread by insect vectors from other crops or weed hosts in the vicinity even though the stock plants are originally virus-free. Unfortunately, some viruses are brought into the nursery through plants imported from foreign sources or through exchange between domestic nurseries and/or retailers.

Nursery crops are susceptible to a broad range of viruses, some of which have been identified and some of which are not yet understood. As more and more improved varieties of nursery crops are introduced and grown for sales, knowing what viruses are prevalent in bedding and flowering plants through science-based knowledge and clinically verified diagnostics will help in the production of healthy nursery crops. In addition, availability of science-based information about different viruses prevalent in nursery crops in Washington State and the range of symptoms caused by viruses in different plants under the prevailing environmental conditions in the state will help to develop guidelines for effective monitoring and prevention of virus diseases and to promote increased awareness of virus disease problems among nursery personnel.

Therefore, the principal goal of this proposal is to study virus disease problems impacting perennial nursery crops in Washington State and provide technologies and information for Nursery industry personnel and WSDA to identify and control those diseases. The main objectives of this project are to (i) characterize viruses of economic and quarantine significance to nursery crops, (ii) develop high throughput diagnostic methods for reliable detection of viruses, and (iii) develop awareness programs for the benefit of nursery industry personnel and WSDA.

**Methods of research:****i. Characterization of viruses of major significance to nursery crops**

In a preliminary reconnaissance study of nursery plantings in Eastern Washington during the summer of 2006 (names of nurseries are withheld for confidentiality), we observed symptoms indicative of viral infections in *Monardia sp.* and *Coreopsis sp.* Initial testing of these samples at WSU-IAREC, Prosser, by Enzyme-linked immunosorbent assay (ELISA) has indicated that several viruses could be present as mixed infections. Since plantlets from these perennials are supplied to different wholesale/retail customers within and outside the state, there is a risk of spreading these viruses throughout the country and causing economic losses to the nursery industry. Spread of these viruses to other regions of the country has implications for quarantine regulations and could tarnish the reputation of the Washington nursery industry.

We will conduct detailed studies on viruses found in *Monardia sp.* and *Coreopsis sp.* using different virological and molecular biological methods to precisely identify the virus(es) present in these plants. Leaf extracts from *Monardia sp.* and *Coreopsis sp.* will be mechanically inoculated on to a set of indicator host plant species to separate these viruses and maintained in the greenhouse to protect from external infection. The plants will be monitored at regular intervals for symptom development and tested by ELISA to identify the virus(es). In addition, specific parts of the genomes of these viruses will be amplified by Polymerase Chain Reaction (PCR) technique and the amplified fragments will be cloned and nucleotide sequence determined using standard molecular biology techniques. The sequence data will be compared with genome sequences of other viruses documented in the GenBank to validate ELISA results and precisely identify the type of virus(es) encountered in *Monardia sp.* and *Coreopsis sp.*

**ii. Diagnostic methods for reliable detection of viruses**

The inability to accurately diagnose plant viruses in perennial nurseries could result in misrepresentation of the economic significance of a particular virus. Our goal is to help nurseries and regulatory agencies take accurate aim at these viruses through reliable and sensitive diagnostic methods. Although ELISA has been a preferred detection method due to its simplicity and effectiveness, it has failed on occasion due to low virus titer, or inhibitory effects of various compounds present in sample extracts. Molecular testing by RT-PCR is often able to detect viruses at much lower concentrations than ELISA. This is critical in nursery perennials, since many times symptoms are not clearly expressed due to low virus titer. For this purpose, we will initially develop RT-PCR assays for accurate detection of virus(es) that we have found in *Monardia sp.* and *Coreopsis sp.* The RT-PCR assay will be validated using field samples collected from nurseries. The incidence of these viruses in different varieties of *Monardia sp.* and *Coreopsis sp.* will be estimated by PCR assay.

**iii. Develop awareness programs for the benefit of Nursery industry and WSDA.**

The results obtained from this project will be disseminated through different avenues for the benefit of Nurseries, WSDA, and other stakeholders of the nursery industry to facilitate safe movement of plant materials and for better preparedness when an exotic virus is accidentally introduced into the state. These efforts include one-on-one meetings with nurseries during field visits and presentations at industry meetings to highlight research findings, and publishing articles in nursery-related magazines and peer-reviewed scientific journals for increased awareness among stakeholders.

**Expenditure Breakdown:**

(Please include salaries, supplies, travel, etc.)

**Salaries<sup>1</sup>**

Research Associate	:	\$3,776
Time slip	:	\$2,375

<b>Supplies<sup>2</sup></b>	:	5,000
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<b>Travel<sup>3</sup></b>	:	1,720
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**Employee benefits**

Research Associate (@36%)	:	\$1,359
Time slip (@11.5%)	:	\$ 273

<b>Total</b>	:	<b>\$14,503</b>
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<sup>1</sup>Salary (one month) for a Research Associate to conduct proposed research and coordinate lab and field research activities. Time-slip (250 hrs @9.50/hr) to maintain plants in the greenhouse and assist in sample collection in the field and preparation of sample extracts for testing by ELISA and PCR.

<sup>2</sup>Research consumables and lab disposables for use in virus characterization and molecular analyses, carrying out diagnostic methods, and greenhouse materials (pots, soil and fertilizer) to maintain plants.

<sup>3</sup>Vehicle charges (@ \$0.36/mile) for visiting nurseries to collect samples and attend one industry annual meeting (room, per diem and transport)

**The information requested on this page will have a direct bearing on whether your research request is approved or denied. Letters of support by the industry are also encouraged.**

**Note: Funding is not available for general overhead cost.**



# Northwest Horticulture

951 Allison Road • Mabton • WA 98935  
Telephone 509-894-5012 • Fax 509-894-5397

December 12, 2006

Mr. Tom Wessels  
Plant Services Program Manager  
P.O. Box 42560  
Olympia, WA 98504-2560

Dear Mr. Wessels,

I am writing to support the research proposal submitted by Dr. Naidu Rayapati for Nursery Research Funding "Characterization and detection of viruses impacting the nursery industry in Washington State".

Our company is one of the largest producers of ornamental perennial plants in the nation (we produce about 10% of the flowering perennials in the U.S.). We have locations on both sides of the state, in Mabton and Mt. Vernon, and we are the largest employer in the town of Mabton.

We are very concerned about the consequences of plant virus infection. In the past, virus diseases have devastated certain segments of the annual ornamental industry, and we want to make sure this doesn't happen with perennials. Because we ship plants to all parts of the US and Canada, it is critical that we maintain clean stock. Although we screen our plants for the 14 most common ornamental viruses, other unidentified viruses may be affecting our ability to grow some varieties. Dr. Rayapati's work in identifying viruses and developing detection methods will give us a head start in eliminating problems that may surface in the future. Also, this work may give us insight into the reasons certain varieties are difficult to propagate and grow.

We hope your committee will decide to fund this research, which will be valuable to perennial growers throughout the state.

Sincerely,



Anne Morrell, Ph.D.  
Stock Plant Manager